The protective effect of mycorrhizal fungi on the host plant

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The favourable effect of mycorrhizal fungi on the plant-partner has been explained by the possibility of a protecting influence (Hacskaylo 1957, Zak 1964, Ritter and Lyr 1965 and others). Various factors which may be taken into account are discussed by Zak (1964). According to whom mycorrhizal fungi are able to protect the roots of host plants against pathogens in the following ways: a) they utilize superfluous carbohydrates in the close vicinity of roots and this area then looses its attraction for pathogens, b) they provide a physical barrier, c) they secrete antibiotics, d) they support the growth of favourable rhizosphere microorganisms in the vicinity of roots. There are only few experimental foundations for these opinions.

Santoro and Casida (1962) tested the antibiotic activity of 5 species and Šašek and Musílek (1967) 17 species of mycorrhizal fungi against commonly used test-microorganisms. In spite of the fact that several active strains have been found, their number and activity was lower as compared e.g. to the previously studied wood-destroying fungi. The antibiotic activity of cultures of mycorrhizal fungi was not increased when combined with seedlings of the host plant (Šašek and Musílek 1967a).

Krywolap and Casida (1964) studied the antibiotic activity of the fungus Cenococcum graniforme. All 7 strains studied formed the same antibiotic. In a further paper (Krywolap, Grand and Casida 1964) the authors succeeded in extracting an antibiotic with the same characteristics from mycorrhizas, seedlings and some organs of several mycorrhizal trees. On the basis

of these results the authors discussed the fact that forest--trees are more susceptible to fungal than bacterial attack. They see the possible explanation of this situation in the fact that Cenococcum graniforme is a frequent mycorrhizal fungus which produces mainly an antibacterial antibiotic.

Some positive results of the study of growth inhibition of parasitic fungi by cultures of mycorrhizal fungi (Šašek and Musílek 1967a) led us to examine, these relationships during the in vitro infection of seedlings. Therefore, pine seedlings were cultivated under sterile conditions together with mycorrhizal and parasitic fungi and the protection of seedlings by various species of mycorrhizal fungi were studied.

Protecting influence of mycorrhizal fungi on seedlings infected by fungi causing their damping-off

Using the method of polyurethane floats (see Šašek and Musílek 1967a) we cultivated pine seedlings together with following species of mycorrhizal fungi: Amanita citrina, Clitopilus prunulus, Lactarius helvus, Rhizopogon roseolus, Russula fragilis, Scleroderma aurantium, Suillus bovinus (4 strains), Suillus luteus (5 strains), Suillus variegatus (4 strains), Tricholoma albobrunneum and Tricholoma saponaceum (2 strains). After 20 days of cultivation cultures of the parasitic fungi Rhizoctonia solani, Pythium debaryanum, and Fusarium oxysporum were added to respective flasks. The experiments were terminated after 2 weeks of simultaneous cultivation, when the control seedlings were completely destroyed by the parasite.

All strains of Suillus bovinus and 1 strain of Tricholoma saponaceum showed a protective effect against Rhizoctonia solani. Cultures of Amanita citrina, Russula fragilis, Scleroderma aurantium and Tricholoma saponaceum protected the seedlings against Pythium debaryanum. Against Fusarium oxysporum, Scleroderma aurantium and Tricholoma saponaceum were effective (see Table I and Fig. 1).

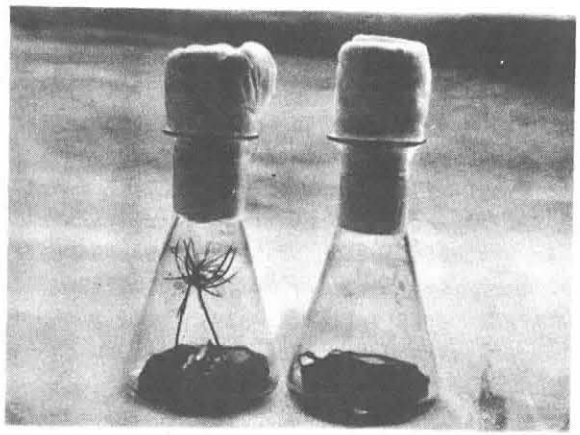

Fig. 1
The protective influence of the mycorrhizal fungus Tricholoma saponaceum against the effect of the parasitic fungus Pythium debaryanum (control on right side).

Table I

List of mycorrhizal species interfering with the effect of damping-off fungi

Mycorrhizal Fungus	Parasitic Fungus		
	Rhizoctonia solani	Pythium debaryanum	Fusarium oxysporum
Suillus bovinus, strain I-III	+		
Tricholoma saponaceum	+	+	+
Amanita citrina		+	
Lactarius helvus		+	
Russula fragilis		+	
Scleroderma aurantium		+	+

+Mycorrhizal fungus protected the seedlings against infection.

The influence of mycorrhizal fungi on infection by Lophodermium pinastri

The eventual protection of some cultures of mycorrhizal fungi against the effect of Lophodermium pinastri was determined by the use of sterile sand cultures of seedlings and mycorrhizal fungi in vitro (Melin 1925). On the basis of experimental results with simultaneous cultivation of mycorrhizal fungi with Lophodermium pinastri (Šašek and Musílek 1967a) the following species of mycorrhizal fungi which slowed growth of the parasite were chosen: Lactarius helvus, Russula fragilis, Scleroderma aurantium, Suillus luteus and Tricholoma saponaceum.

After 6 weeks of simultaneous cultivation of seedlings and mycorrhizal fungi the culture of Lophodermium pinastri was added. The harmful influence of the parasite was shown to be atypical; the parasitic fungus grew abundantly during one month and a mechanical suppression of seedlings took place without any specific attack.

To determine whether the high humidity of air inside the cultivation flasks was the cause of this atypical course of infection, the experiments were repeated in an apparatus for simultaneous cultivation of fungi and seedlings under decreased relative humidity (Fig. 2). We used a method similar to that of Plunkett (1956) with a desiccator as the cultivation vessel. The desiccator contained several dishes with prepared sand (Melin 1925) and nutrient solution (Melin 1936), (250 g of sand: 45 ml of solution). The inlet air was dried with anhydrous $CaCl_2$, sterilised by a glass-wool filter, and its flow measured by a flow-meter. Movable tubing connected with the supply bottle enabled the watering of individual dishes. For measuring the volume of water added a pipette was placed between the watering-tube and supply bottle. An attached

hygrometer was used to measure the relative humidity of the outlet air. Under the conditions described and using an air flow of 4 l/min. the relative humidity of air inside the desiccator was 60%. To maintain the respective humidity of the sand it was necessary to add to every dish 13 ml of solution every 4 days. To avoid a superfluous concentration of nutrients as a result of frequent watering, the supply bottle contained the original nutrient solution diluted with distilled water (1:10). The apparatus was steam sterilised. The ground glass surfaces were treated with silicon grease. The cultivation vessel was placed near a window and, moreower, during the day illuminated by an 100 W bulb from 20 cm distance.

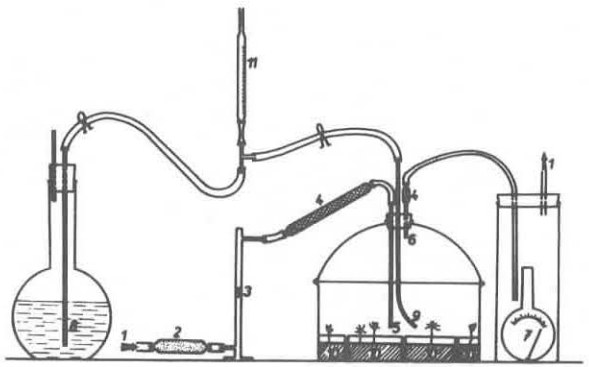

Fig. 2
Apparatus for long-term cultivation of seedlings under sterile conditions with decreased relative humidity
^{1}air-flow, $^{2}CaCl_2$, ^{3}flow-meter, ^{4}glass-wool filter, ^{5}air inlet, ^{6}air outlet, ^{7}hygrometer, ^{8}supply bottle with nutrient solution, ^{9}watering-tube, ^{10}dishes with sand, ^{11}pipette for dosage of solution.

The seedlings were cultivated for 6 weeks together with mycorrhizal fungi at 100% relative humidity. After the additional inoculation with Lophodermium pinastri the relative humidity was decreased to 60% in the course of 14 days, and was kept at this level until the end of the experiment. During three further weeks of cultivation the parasite again suppressed unspecifically the seedlings. During 8 months cultivation no sign of seedling disease caused by Lophodermium pinastri was apparent when inoculated with Tricholoma saponaceum and Scleroderma aurantium.

DISCUSSION

The results achieved in laboratory experiments, i.e. the low antibiotic activity of mycorrhizal fungi (Šašek and Musílek 1967), their small effect on the growth of parasitic fungi (Šašek and Musílek 1967a), and the few favourable results in the protection of seedlings against the parasites cited in this paper, demonstrates the protecting effect of mycorrhizal fungi is not the main factor in the favourable influence of mycorrhizal fungi on the host plant. On the basis of various contradictive indications in the literature on the mycorrhizal relationship and on the basis of results achieved, we concluded that it is probably not possible to explain relation between fungi and plant in a simple manner. The association is affected by environmental factors (humidity, light, temperature, soil composition, soil microflora, etc.), influencing the plant and the fungus in a complex manner and thus adjusting the equilibrium of their relation. Moreover, the resulting effect on the development of the plant depends evidently also on the species of the respective mycorrhizal fungus. In other words the favourable influence of fungi on the plant can be manifested in various ways; by enabling greater intake of water and salts, by providing the possibility to obtain inorganic compounds from previously inaccessible sources, by supplying organic nutrients, vitamins, growth-promoting substances or compounds of an antibiotic character. The relation

between the fungi and the plant depends on the condition of environment to such a degree that each of the factors cited above can become a dominant one under certain individual conditions.

Although the results achieved do not suggest any fundamental significance of the protecting influence in the mycorrhizal relation it is not possible to overlook the antibiotic formation detected in some mycorrhizal fungi, as well as their inhibition influence on the growth of some parasitic fungi and the protective influence of several species of mycorrhizal fungi on pine seedlings under laboratory conditions. We can assume that also under certain natural conditions the protecting influence of some species of mycorrhizal fungi could manifest itself as one of the favourable factors in the fungus-plant relation.

It is necessary to point out that experiments with seedlings have taken place in vitro. To evaluate the experiments with Lophodermium pinastri it is necessary to verify these results under natural conditions using natural infective material in order to obtain the normal course of the respective disease. On the other hand in experiments with fungi causing damping-off of seedlings the experimental conditions given were extremely favourable for the parasitic fungi (the parasite destroyed the control seedlings very soon; under conditions of high air humidity the seedlings were destroyed even by harmless imperfect fungi, such as Penicillium). Therefore, it cannot be excluded that under conditions less favourable for the parasite the protective effect could be promoted also by other mycorrhizal fungi.

The confirmation of our results under natural conditions would mean a realistic basis for biological protection of seedlings in nurseries. Then it would be possible to add to the soil in nurseries those cultures of mycorrhizal fungi which showed the protecting influence. The possibility of

transfer of pure cultures of mycorrhizal fungi to the forest soil has been proved; moreover, it was found that several fungal species may be involved in the mycorrhizal formation on the roots of the one seedling. Therefore, there is a possibility of artificially directing the formation of mycorrhizas most adequate to the actual conditions of the seedlings. We have to point out the great economic importance of massive destruction of pine seedlings in nurseries as a result of damping-off and pine needle infection by Lophodermium pinastri.

The mechanism of protecting influence does not seem to be uniform. With some fungal species the production of antibiotics could come into consideration (from the species studied, Tricholoma saponaceum and Rhizopogon roseolus - see Šašek and Musílek 1967b), with other species (e.g. Suillus bovinus) the competition for the essential nutritients with parasitic fungi in the soil. This possibility is supported by the results achieved by Dominik (1961) who found that in the soil, where previously agricultural plants were grown, the pine seedlings have been destroyed by fungi causing their damping-off. In his opinion a balance is necessary between mycorrhizal fungi and other organisms in the soil before the sowing of seeds. Our experimental results support this opinion, for instance when the Suillus bovinus was cultivated together with the seedlings, the growth of additionally inoculated Rhizoctonia solani was less intensive and the seedlings were not destroyed. However, the test of antibiotic activity of Suillus bovinus has not shown any effect against Rhizoctonia solani. It is logical that if some mycorrhizal species show a protecting influence on seedlings this fact is to be based on the effect of the mycorrhizal fungus on the parasitic one in the soil and not on the formation of any physical barrier on the surface of roots, because the attack of fungi causing the damping-off takes place at an early stage before mycorrhiza formation.

SUMMARY

We have studied a group of 11 species (22 strains) of pine mycorrhizal basidiomycetes in connection with seedlings infected artifically by parasitic fungi.

A protecting influence has been proved only with the mycorrhizal species Amanita citrina, Lactarius helvus, Russula fragilis, Scleroderma aurantium, Suillus bovinus and Tricholoma saponaceum.

These results together with our previous knowledge about the small antibiotic activity of mycorrhizal fungi demonstrate the protective influence not to be the main and general function of mycorrhizal relation, though it could operate in some individual cases.

References

Dominik T. 1961: Prace Institutu bad. Les. 210:103-162.
Hacskaylo E. 1957: Ohio J.Sci. 57, 6:350-357.
Krywolap G.N., Casida L.E.,Jr. 1964: Can.J.Microbiol. 10, 3:365-370.
Krywolap G.N., Grand L.F., Casida L.E.,Jr. 1964: Can.J. Microbiol. 10, 3:323-326.
Melin E. 1925: Untersuchungen über die Bedeutung der Baum-mykorrhiza. Jena, G.Fischer, 152 pp.
Melin E. 1936: Handbuch biol.ArbMeth. 11,4,2:1015-1108.
Plunkett B.E. 1956: Ann.Bot.N.S. Lond., 20, (80):563-586.
Ritter G., Lyr H. 1965: Plant Microbes Relationship, Proc.Symp. of Relat. between Soil Microorg. and Plant Roots, Prague 1963: 277-282. Publ. ČSAV, Praha.
Santoro T., Casida L.E., Jr. 1962: Can.J.Microbiol. 8:43-48.
Šašek V., Musílek V. 1967: Fol.microbiol., in press.
Šašek V., Musílek V. 1967a: České mykol., in press.
Šašek V., Musílek V. 1967b: Fol.microbiol., in press.
Zak B. 1964: Ann.Rev.Phytopath. 2:377-392.